Table 3: CE Usage for Process and Product Characterization

Session 1:
Facilitator:
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Session 2:
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Scope:
Capillary electrophoresis (CE) methods have become an increasingly essential part of the analytical control strategy throughout the biopharmaceutical industry. Automated instrumentation, powerful separation efficiency, low sample requirements, and fast analysis times have made CE a great analytical tool for drug characterization. Various separation modes such as cIEF, CE-SDS, CZE, and CE-MS all have their own unique challenges when performed on different instrumentation and platforms. The goal of this roundtable session is to discuss CE usage for process and product characterization and to exchange common solutions across the industry.

Questions for Discussion:
Current Applications and Opportunities:
1. Which CE methodologies are regularly used for process and product characterization (e.g. CE-SDS, iCIEF, CZE, CE-glycan etc)?
2. What instrument platforms regularly accompany these methods?
3. What CE applications and Instruments are not typically covered?

CE Technology Experience:
1. What is your experience level with CE?
2. What is the range of analysts' experience who run CE in your lab?

Challenges for CE methods in process and product characterization:
1. What are the common causes of error?
2. How frequently are issues observed?
3. What do you do to reduce or eliminate these sources of error?

CE method transfer:
1. How are CE methods transferred to testing sites?
2. How is troubleshooting incorporated into the site transfer process?

Discussion Notes:
Session 1:
Current Applications and Opportunities:
- Which CE methodologies are regularly used for process and product characterization (e.g. CE-SDS, iCIEF, CZE, CE-glycan etc)?
    - In addition to above mentioned CE methods, CE-MS methods, Affinity CE and Microchip CE are used.
- What instrument platforms regularly accompany these methods?
    - Ion exchange platforms like SCIEX PA800, Agilent CE System, Protein Simple iCE System (Maurice) and 908 Devices
- What CE applications and Instruments are not typically covered?
    - Affinity CE is not used for binding potency
    - 2-D CE is not available while 2-D gels for HCP detection.
    - Problem of CE-LIF: toxic reagent (KCN)
    - Testing for charge profile of ADC is not stability indicating due to peak slit by deamidation.

CE Technology Experience:
- What is your experience level with CE?

    - In addition to above mentioned CE methods, CE-MS methods are used for PTM modification and localization, characterization study using forced degradation samples, etc.
- What is the range of analysts' experience who run CE in your lab?
    - Identification of CQAs, process development and release and stability testing
    - CE-SDS, iCIEF and affinity CE can be used for binding kinetics and formulation study.
    - Microchip CE for clone search, process characterization and process comparability
    - Fractionation by iCIEF for characterization of minor peaks or impurity cleanup for peptide products

Challenges for CE methods in process and product characterization:

- What are the common causes of error?
    - Baseline problem with UV for CE
    - Ghost peaks in CE-SDS
    - Sensitivity problem in iCIEF
    - Peak profile shifting due to capillary coating problem
    - Column batch inconsistency and reagent quality (EACA, Sod sulfate, etc)
- How frequently are issues observed?
    - Up to 30% failure rate in system suitability for old molecules
- What do you do to reduce or eliminate these sources of error?
    - Training, protocol revision and rerun in QC

CE method transfer:

- How are CE methods transferred to testing sites?
    - Involvement of SMEs for testing onsite
- How is troubleshooting incorporated into the site transfer process?
    - Robust training package
    - Documentation

<u>Session 2:</u>

- Why people selected this roundtable:
    - Want to learn how to practice CE in industry
    - Want to learn more about other people's usage
    - Want to learn about CE to potentially replace LC methods
    - Want to learn how customers are using their products

- Current Applications and Opportunities:
    - What methods do you use and what do you use them for?
        - CE-SDS to monitor size variants and fragmentation, especially with stability samples
        - CE-glycan to monitor glycosylation which could impact potency
        - cIEF to monitor charge variants
        - CZE for identity testing
            - Others don't use it much but could maybe develop it in the future. Since you don't have to modify the molecule, the simpler sample prep could be a strength
        - CE-MS is still under development – there are a few different formats available and if the method works it could be adopted but it is not in common practice currently for QC, only in early stage
    - What instruments are most useful?
        - PA800+ for CE-SDS (size heterogeneity) and CE glycan (QC lab)

        - LabChip can be used if you want higher throughput – used more to support early process development and use PA800 for later process development testing.
        - ICE3 and Maurice instrument for cIEF to monitor charge was only option used at table discussion.
        - Note: process and product characterization deals with monitoring anything from cell culture to final product
        - Challenge: "in process samples may contain high salt and low protein concentration and as result we use ion exchange method. Alternatively, could buffer exchange samples with high salt to use CE method but this extra step is not preferred."
            - 908 Devices CE-MS instrument has the injection cross-junction and is not as sensitive to sample matrix
            - LabChip also has the injection cross-junction, but it can still be sensitive to the matrix

    - What detectors do you use with CE?
        - LIF or UV/PDA with CE-SDS (both options used at table discussion)
        - Native fluorescence with cIEF (Maurice)
        - No current demand for ECD

    - What are you looking to gain by having a CE method in process characterization?
        - Speed, high throughput
        - Clearance monitoring – with LC methods some excipients and impurities take a long time to elute but they are charged – CZE could potentially be used to speed up such methods

- Challenges for CE methods in process and product characterization:
    - Need buffer exchange or desalting before CE-IEF
    - Glycans aren't commonly analyzed using CE, typically LC methods are used
    - Common causes of error
        - Instrument error
            - capillary window breaking, end snapping off
            - inconsistent maintenance
                - possible remedy: greater attention to cleaning every part, not just the easy to access external ones
            - poor capillary performance
                - possible remedy: cartridge counting to monitor the number of injections a capillary has had (like LC column counting) – note: more newer systems are able to track this (LabChip, and Maurice ProteinSimple)
            - lot-to-lot variability of CE-SDS cartridges due to variations in the bare silica capillaries
                - possible remedy: conditioning after each run
        - Human error and inconsistent training
            - possible remedy: train with examples of troubleshooting so you know what to expect
            - possible remedy: Empower control with PA800 (e.g. build one sample table instead of inputting sequence into two systems)
            - possible remedy: precut capillaries
        - Other remedies

                - Collect pressure, voltage, current channels to help understand data sets
                - Develop good procedures to decide when to have data verified or not

    - Troubleshooting with large sample quantities
        - Methods
            - cIEF use Maurice with a 96-well plate and onboard mixing, automated sample prep, but sensitivity can be lost with time
            - Include standard controls across an assay for a large data set, especially with early research
            - CE-SDS: the only automated part is sample dilution, everything else is manual with systems of criteria for troubleshooting
            - There is interest in using CE for high throughput glycan analysis
        - Data Analysis
            - Unstable UV baseline makes it difficult to use automatic integration for CE-SDS in 32 Karat
                - Possible remedy: transfer data to Empower to customize integration; for QC it would be easier to review if they were using Empower. Empower driver from Sciex will be helpful.
            - Issues in method transfer in the mid-MW range and difficulty reproducing ghost peaks

- In an ideal world, what would you want to exist for use in process and product characterization?
    - Platform methods
        - Will be easier for size heterogeneity than for charge because it's hard to have one cIEF method that is optimized for molecule with low or high pI values.
    - Automated data analysis with minimum manual integration time
    - Preparative charge variant instrumentation
        - People used to use off gel for this, but it's not on the market anymore
    - Instruments with multiple capillaries to ensure the same sample stability throughout
        - This exists for glycans (C1HT100) to do 12 at a time, but doesn't exist for proteins yet
    - More automation, e.g. robots manipulating plates instead of manual capping and moving of vials (possible issue: evaporation)

- What other opportunities are there to use CE in product characterization?
    - It is difficult to separate DARs on HIC – micelle tagging electrophoresis could be interesting
    - Drug antibody conjugates using CE (cIEF)